Evaluation of Shear Bond Strength of Composite Resin Veneers and Nickel-Chromium Alloy - An Invitro Study

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Abstract

Objective: This study measured and compared the shear bond strengths of composite resin veneer to Ni-Cr alloy with mechanical, chemical and mecanochemical retention methods.

Materials & Methods: A total of 96 base metal Ni-Cr alloy cast specimens with meshwork, beads for mechanical, SR Link, Alloy Primer for chemical and combination of these two form mecanochemical bonding were prepared. Composite buttons were built and cured on the specimens and tested for shear bond strength. The study was categorized based on the mode of retention into eight groups of twelve specimens each. Half of the samples from each group (6) were tested before thermocycling and the rest half were tested after subjecting them to 500 cycles of thermocycling with a temperature variation from 5°C to 55°C.

Results: The bond strength of a light cure composite material was higher in mecanochemical-meshwork+SRLink, beads+SR Link, followed by beads, SR Link, and the least was observed in Alloy Primer. Thermocycling produced a significant decrease in bond strength in most of the groups.

Conclusion: The use of meshwork+Alloy Primer combination and the beads or SR Link used either alone or in combination produced higher shear bond strength of the composite to the metal alloys.

Keywords: mechanical retention, metal alloys, primers, resin composite, shear bond.

I. Introduction

The esthetic quality of a restoration may be as important to the mental health of the patient as the biological and technical qualities of the restoration are to his physical or dental health. Today, esthetic considerations are the primary factors for seeking dental treatment. Another basic requirement of the restoration is that, it should be strong enough to resist the functional load which could be accomplished by using metal. Both these functional and esthetic requirements can be solved by veneering the metal base of a restoration with esthetic materials like porcelain or composite resin.

These resin veneers can be retained on the metal casting by mechanical, chemical or a combination of both methods. Mechanical bonding relies on macro mechanical or micro mechanical treatment of metal alloys. Beads, nail heads, gauze, peripheral undercuts, wire loops, nylon bristles, stubs, serrations and mesh are considered macroretention devices. Some of these require additional facial thickness or excessive facial reduction to accommodate the retention and have been associated with over contouring and space problems. Micro mechanical means of retention include sand blasting or electrochemical etching. They improve retention between the alloy and the resin by eliminating oxides and greasy materials from the alloy surfaces, thereby creating a fine roughened surface and increasing the surface area, all of which enhance mechanical or chemical bonding. With the exception of air abrasion most techniques are associated with expensive equipments, technique sensitivity and harmful chemicals.

Furthermore in mechanical retention, the absence of chemical adhesion subjects the interface to breakdown over time. It would be advantageous to find an uncomplicated method of achieving a chemical bond between the veneering resin and the metal.
Since Rochette first described the silane coupling method to bond resin to a metal framework, various chemical methods have emerged to improve the bond strength between resins and metals. The chemical bonding of resin veneers eliminate the need for these bulkier macro retentive features. The esthetic veneering is also improved because there is more available space for a dentin/enamel layer. Recent chemical bonding techniques for metal alloys can be either a surface modification technique that creates a thin layer of inorganic, organic and metallic material on the substrate of the metal alloys like Silicoater, Rocatec, Kevloc systems, tin plating, Ion coating, or the use of an adhesive monomer targeting direct chemical bonding between the metal alloys and resin components. The main components that are used in the metal primers are VBATDT: 6-(4-vinyl benzyl - n-propyl) amino-1, 3, 5 triazine 2, 4 dithione, MDP: methacryloyloxydecyl dihydrogen phosphate, MEPS: methacryloyloxyalkyl thiophosphate derivative and MTU-6: 6: methacryl olyxyhexyl 2 thiouracil 5- carboxylate.

II. Aims& Objectives
This in-vitro study was taken to evaluate the bond strength of laboratory composite resin-metal interface using mechanical, chemical and mechanochemical methods with the following aims:
1. To determine the bond strength of mechanical methods -beads and meshwork pattern
2. To determine the bond strength of chemical methods – SR Link and Alloy Primer
3. To determine the bond strength of mechanochemical methods combination of the above two
4. To determine the bond durability using thermocycling method.

III. Materials& Methods
The materials selected for this study are laboratory cured composite resin (Solvent Resistant-SR Adoro) and Ni-Cr alloy (CB-80). Beads and meshwork wax pattern were used for mechanical retention, and metal primers SR Link, Alloy Primer for chemical retention. The above two methods were combined for mechanochemical retention. The study was categorized based on the mode of retention into eight groups of twelve specimens each. So a total of 96 specimens were prepared. Half of the samples from each group (6) were tested before thermocycling and the rest half were tested after subjecting them to thermocycling.

3.1 Grouping the Samples
Group I - Beads
Group II - Meshwork
Group III - SR link
Group IV - Alloy Primer
Group V - Beads + SR Link
Group VI - Beads + Alloy primer
Group VII - Meshwork +SR Link
Group VIII - Meshwork +Alloy Primer

IV. Methodology
4.1 Fabrication and Surface Preparation of Resin Samples
A 96 cylinder shaped specimens of 10mm in diameter and 4mm height were prepared with cold cure acrylic resin using a metal die. For the bead retained groups (Group I, V, VI) polymer beads of 400µ - 600µ(SR Macro retention beads) were used to provide mechanical retention. Approximately 50 beads per sample were placed on the bonding surface using a thinnest moist probe and cyanoacrylate adhesive (Fig-1a). For the meshwork retained groups (Group II, VII, VIII ), a 1cm × 1cm square of preformed meshwork wax pattern was cut and adapted over the surface of the resin samples(Fig-1b). No surface modification was done for both the chemicalgroups (Fig –1c) (Groups III, IV).

4.2 Spruing & Investing
A flexible silicone-casting ring was used for the investing purpose. Six acrylic resin samples were arranged in a circular pattern on the crucible base; the patterns were approximately 6mm from the top of the ring and 3 mm from the wall of the casting ring. They were separated from each other by a distance of 3mm (Fig-2). Surfactant was applied over the patterns and left to air dry. A phosphate-bonded investment (Flexivest-IvoclarVivadent) was mixed with silica sol in the proportion of 160 grams of powder to 35 ml of liquid using a vacuum mixer. The silicone ring with the resin samples was placed over a mechanical vibrator and then invested.
4.3 Casting& Finishing
Casting procedures were done using a spring-loaded centrifugal casting machine. The Ni-Cr alloy pellets (Dentsply-Sankin) were melted with a gas - oxygen torch from a distance of approximately 3 cm and the entire casting procedure was completed approximately within a minute. Castings were divested and sandblasted with 100 grit aluminium oxide (Fig-3). The cast samples were mounted in the center of the acrylic resin block, parallel to the cross section of the block, so as to enable the application of testing force in the correct direction (Fig-4a). The bonding surfaces of the mounted samples of all the groups were finished using a non-recyclable 250 microns aluminium oxide at 3 bar pressure, held at a distance of 5mm from the nozzle. The metal primers SR Link and Alloy Primer were applied to the sandblasted surfaces of the samples of chemical and mechaanochemical groups using a brush and left undisturbed for 3 minutes.

4.4 Fabrication of Composite Buttons
A piece of circular adhesive tape with a central hole of 5mm diameter was placed on the bonding surface of the cast samples to define the area of bonding (Fig-4b). The first opaque layer (SR Adoro) is applied thinly using a brush in the defined bonding area, and precured for 20 seconds using the Quick curing light (wavelength 400-500nm). The second opaque layer was applied till the metal framework was completely covered with the opaquer. Then it was precured for 20 seconds with the Quick light-curing unit and then polymerized in the Lumamat 100 for 11 minutes. This tempering furnace combines light with heat to maximize the polymerization there by improving the mechanical properties of the veneering composites. After curing the opaque layer in Lumamat 100, superficial sticky surface was removed using a disposable sponge. Then a Teflon ring (4mm length, 6mm inner diameter, and 1mm wall thickness) was placed centrally over the opaque coated test area (Fig-4c). The ring was filled with dentinal resin; each resin layer (2mm thick) was polymerized separately totaling 4mm in thickness. The polymerization was carried out according to manufacturer’s instruction (each segment was precured for 20 seconds (Fig-5a), followed by final curing in Lumamat 100). The final tempering process was performed at 105°C for 25 minutes in the Lumamat 100 (Fig-5b) for optimum polymerization so that the optimum physical properties of the composite resin can be achieved.

4.5 Thermocycling Procedure
To evaluate the durability of bond strength between the composite resin and metal alloy surface, thermocycling procedure was performed. The total samples were split into two halves (48 each). The shear bond strength of one half was tested without thermocycling (TC 0). The rest of the samples (48 samples) were subjected to manual thermocycling. In this procedure the samples were exposed to temperatures of approximately 5°C and 55°C alternatively with an immersion time of ten seconds in each. This immersion in cold and hot temperatures for ten seconds in each completes one cycle. 500 cycles (TC 500) were completed and then the samples were subjected to testing for shear bond strength.

4.6 Shear Bond Testing
Shear bond strength of the composite was determined by using Lloyd’s Universal Testing Machine (Floor type) with a crosshead speed of 0.5 mm per minute. The Teflon tubes were removed and the samples were positioned in the vise, in such a way that the upper shearing blade was flush on the metal surface and perpendicular to the composite cylinders(Fig-6). The maximum load required to fracture a sample divided by the bonded area was recorded as the shear bond strength of that particular sample.

V. Results
The peak rupture load at which the bond failure occurred was recorded in Newtons and the bond strength was calculated using the formula, the breaking Load at which debonding occurs (N) divided by surface area of the bonding surface (mm2). All values of the shear bond strength between the metal and the composite veneering resin were calculated according to the surface area of the specimens and expressed in MPa (N/mm2).

5.1 Shear Bond Strength Before Thermocycling:
Among the eight groups tested without thermocycling (TC 0), group VII (Meshwork+ SR link) exhibited the highest shear bond strength of 13.15MPa and group IV (Alloy primer) showed the lowest shear bond strength of 6.13MPa. The shear bond strength of groups tested are given in decreasing order as follows VII, V, I, III, VIII, VI, II, IV. The bond strength found with meshwork + SR Link is significantly higher than that of the other groups (P < 0.000). The combination of beads and SR Link produced significantly higher bond strength than when either of them was used individually. The combination of Alloy Primer with mechanical retention (beads, meshwork) showed significantly higher bond strength than when it was used alone. The meshwork when used along with metal primers showed greater values than when it was used alone.
5.2 Shear Bond Strength After Thermocycling:
The shear bond testing of the eight groups tested after the thermocycling procedure (TC 500), exhibited a generalized decrease in bond strength. The shear bond strength of groups tested given in descending order as follows VII, V, I, III, VIII, IV, II, VI. The highest percentage of decrease in bond strength after thermocycling was found in group VIII (Meshwork+Alloy primer - 23.07 %) and lowest was in group I (beads-0.8%), group III (SR LINK 0.88%) and group IV (Alloy Primer 1.95%).

5.3 Comparison of Bond Strength Before & After Thermocycling:
The observation of shear bond strength of the group tested before and after thermocycling revealed that, there was a change in shear bond strength ranking. Among the groups tested at TC 0, group IV ranked the last having the lowest shear bond strength, whereas at TC 500, group VI ranked the last in shear bond strength.

VI. Statistical Analysis
The mean values and SDs of the shear bond strengths (MPa) of all the eight retention modes tested at TC 0 and TC 500 are shown bar diagram 1 and table 1. The results were subjected to a two-way analysis of variance to assess the differences between retention methods and thermo cycling conditions.

6.1 Two Way Anova
Analysis of variance will test the significance of differences, among three or more independent groups. When it is believed that two independent factors might have an effect on the test, a Two-way analysis of variance (Two way ANOVA) can be used to test for the effects of the two factors simultaneously. Table 2 presents the factorial ANOVA results of shear bond strength of eight groups. The analysis indicated that the bond strengths were influenced by the type of retention methods used, thermocycling procedure and by the combination of both these methods. Since the P value is less than 0.000, there is a highly significant difference between the groups with regard to shear bond strength. Retention method was the variable with the highest F value and all interactions were significant. The means of bond strengths were therefore compared by using Duncan multiple range tests calculated at 0.05% significance level in table 1.

6.2 One Way Anova
One-way ANOVA and the Duncan multiple range test were performed at a significance level of 0.05% after the two-way ANOVA, to compare the eight different modes of retention. Table 3 & 4 present the one-way ANOVA results of the test groups before and after thermocycling respectively. The analysis indicated that the bond strengths were influenced by the type of retention mode used. Since the P value is less than 0.01, there is a highly significant difference between the groups with regard to shear bond strength.

6.3 Paired ‘T’ Test
When each sample gives a pair of observations, to test for the difference in the pair of values, paired ‘t’ test is used. The values before and after thermocycling are compared by using paired ‘t’ test, which is presented in TABLE 5. From the table 5 we can infer that there is a highly significant difference between TC 0 & TC 500 in groups V, VII, and VIII, where as there is significant difference in groups II & VI and no significant difference in groups I, III, & IV.

VII. Discussion
This study was conducted to evaluate the effect of various retention methods on the bond strength of laboratory cured composite to Ni-Cr alloy, as debonding of the composite resin veneers from the metal surface of the fixed partial dentures is one of the common failures occurring in day-to-day practice. But one of the greatest objections to the use of composites has been the appearance of marginal gaps between the metal surface and the composite due to polymerization shrinkage and difference in the Coefficient of Thermal Expansion. This causes weak bond strength between the two and a change of color due to marginal percolation that affects the prosthesis. To eliminate this problem, many attempts have been made to develop better techniques for bonding composites to dental alloys over the past two decades.

There are several ways of promoting retention of the composite on the metallic surface: micro-mechanical (air abrasion, electrolytic etching, and porous metal coating) macro-mechanical (mesh, beads, rough surface with particles), chemical and a combination of both: mechanochemical methods.

The mechanical method produces retention by increasing the surface area, and by providing locking undercuts. But Kourtis stated that, though mechanical retention produces increased bond strength, it allows micro leakage and requires a high thickness of metal, which can result in over contouring of the restoration. Chemical bonding techniques are preferred over the mechanical retention as they offer certain clinical advantages like reduction in micro leakage and improvement in esthetics.
carboxylic functional monomer used in most of these adhesive opaque resins and promoters were effective in bonding composite resin to metal\textsuperscript{13}. Nabadalung et al\textsuperscript{14}, Watanabe et al\textsuperscript{15}, Taira et al\textsuperscript{16} and several others\textsuperscript{7,12,13,17,18} have reported that adhesive metal primer might be clinically acceptable for bonding a light cured prosthetic composite resin or a denture base resin to a base or precious metal alloy. Ohkubo et al\textsuperscript{19} mentioned in his study that there is a general agreement that, greater bond strengths of resin composites to conventional dental alloys can be achieved when primers are used. Barzilay et al\textsuperscript{20}, Caeg et al\textsuperscript{21}, Tarozzo et al\textsuperscript{22} and others\textsuperscript{3,23} reported that the bond strength was improved in mechanochemical method as a result of the combined chemical adhesion and an increased surface area from the retentive features. Mechanochemical method of retention has the advantages of both of the above methods, but it should be optimized to give the best retention possible.

This study evaluated the effectiveness of eight retention methods i.e., two mechanical (beads, meshwork), two chemical (SR Link, Alloy Primer) and four mechanochemical (combining the above two methods) for bonding composite resin veneer to Ni-Cr alloys. In this study, Shear test was used to investigate the bond strengths because prosthetic appliances in the mouth are mainly subjected to shear forces\textsuperscript{17, 24, 25}

In this present study, the bond strength obtained by beads (9.37 Mpa) is greater than the metal primers: SR Link (9.03 Mpa), Alloy Primer (6.13 Mpa). This result is in agreement with the studies of Keith Moore et al\textsuperscript{23} Luciana et al\textsuperscript{22} in which higher shear bond strength was recorded for the retentive beads than for the chemical bonding techniques. Shue et al\textsuperscript{26} showed that bead retention provided retentive strength equivalent to that of electrochemical etching of the Ni-Cr alloy surface.

In the present study, the use of meshwork did not produce any significant improvement in the bond strength. When both beads and meshwork are considered independently more number of beads could be incorporated within the given area of bonding, thus increasing the surface area and the locking undercuts. In contrast only minimum number of sub squares (4) could be incorporated in the areas of bonding. This might have resulted in the differential rates of bonding between them.

The Alloy Primer used in this study contains VBATDT, and MDP, which is purported by the manufacturer to react with both precious and base metal alloys and the double bonds present in them to copolymerize with the resin monomers. It was anticipated that the use of Alloy Primer would enhance the chemical adhesion between the metal alloy and the composite resin thereby increasing the shear bond strength. However, the results of our study is in contradiction to the other studies that documented satisfactory bond strength using Alloy Primer\textsuperscript{12, 28}. A possible explanation could be due to the difference in the brands of the veneering agent and the type of alloy used. In the present study, base metal alloy was used in contrast with precious metal alloys, which were used in earlier studies\textsuperscript{12, 28}. The difference may also be due to the differences in the compatibility of the functional monomer in Alloy Primer and the initiator for the opaque resin.

The SR link contains phosphate ester and dimethacrylate. The phosphoric acid ester group of the molecule in the bonding agent is a strong acid that reacts with metal or metal oxide and forms a phosphate. The Methacrylate group of the bonding agent reacts with the monomer contained in the opaquers and forms a copolymer. Due to this process a chemical bond with the veneering agent is ensured. The results of the current study showed that the use of SR Link (groups III, V, VII) effectively enhances the bond strength of resin composite to Ni-Cr alloy\textsuperscript{12}.

Matsumura et al\textsuperscript{12} stated on the basis of his experience, a shear bond strength greater than 10 MPa might be necessary for the achievement of clinically satisfactory results using chemical bonding systems. In this study, the chemical bonding systems used were SR Link and Alloy Primer and the shear bond strength obtained with them are 9.03 MPa and 6.13 MPa respectively suggesting that SR Link might serve as an acceptable bonding agent.

By combining the mechanical and chemical methods, the bond strength were significantly improved as shown in table 14 which is in agreement with the previous studies\textsuperscript{20, 22, 25}. In the mechanochemical methods, the groups that were primed with SR Link were found to possess better bond strength than the groups primed with Alloy Primer. The meshwork and SR Link combination produced bond strength of 13.15 MPa, closely followed by bead and SR Link combination 12.6 MPa.

Load and thermocycling are important focuses of dental material testing as they simulate oral conditions. Thermocycling is capable of accelerating the aging of polymeric material; hence deterioration of metal adhesive system can be evaluated within a shorter period. By comparing the bond strength before and after thermocycling or by calculating the percentage of reduction in bond strength after thermocycling, can predict the likely durability of the bonding systems. The shear bond strength of the samples tested with thermocycling and without thermocycling showed a generalized decrease in bond strength in all groups. Among the six modes of retention used, the group VII (Meshwork + SR link) was found to possess superior bond strength before and after thermocycling process. This was followed by group VI (Bead + SR Link). Though both of these mechanochemical methods proved to be better, their clinical application is limited unless they are optimized to reduce over contouring of the veneer surface, which is reported in the literature as their chief
disadvantage. Hence it can be concluded from this study that optimum utilization of a combination of mechanical and chemical retention would give better bonding between the composite resin veneer and the metal framework.

VIII. Summary And Conclusion

One of the common problems that limit the use of resin facings in dentistry is the debonding from the metal surface. Several researches have been conducted and methods improvised to enhance the bond strength. In this in vitro study, mechanical, chemical and mechanochemical methods were experimented and their results analyzed to determine the best retention for light cure composite resins to the Ni-Cr alloy.

The study consisted of 96 samples which were divided into two divisions based on whether they were subjected to thermocycling or not. The two divisions consisting of 48 samples each were grouped into 8 groups depending on the mode of retention used with 6 samples in each group.

Within the limitations of the study, the following conclusions were drawn:
1. The bond strength of a light cure composite material (SR ADORO) joined to a Ni-Cr alloy (CB 80) was improved with the use of Beads for retention.
2. Among the chemical methods used, high bond strength was obtained with the metal primer – SR Link containing phosphate ester and dimethacrylate.
3. In the mechanochemical retention methods, the Meshwork + SR Link combination showed higher bond strength, which was closely followed by Bead + SR Link combination.
4. The use of Alloy Primer did not significantly improve the bond strength when used either alone or in combination with bead and meshwork.
5. While the highest bond strength was seen in Group VII (Meshwork + SR Link), the least bond strength was noted in groups using Alloy Primer.
6. The bond strength obtained with the use of Meshwork and the combination of Beads + Alloy Primer were not significantly different.
7. Thermocycling produced a significant decrease in bond strength in all groups except the Groups I, III and VII. This decrease was highest for Group VIII - 23.07% and least for Group I – 0.8%

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References

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FIGURES

Figure 1: resin samples 2a-beaded, 2b-meshwork adapted, 2c-plain

Figure 2: sprued resin samples

Figure 3: sprued resin samples
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Figure 4a: mounted samples, 4b defined bond area, 4c: teflon ring for compostesss

Figure 5a: quick cure unit  5b: final curing unit

Figure 6: sample after debonding

BAR DIAGRAM 1

![Bar Diagram](image-url)
**TABLES**

**TABLE 1** the mean values and sd of the shear bond strengths (mpa) &duncan grouping of all the eight groups at tc 0 and tc 500, and the decrease (%) in shear bond strength after thermal cycling.

<table>
<thead>
<tr>
<th>Group</th>
<th>TC 0 Mean (SD)</th>
<th>TC 500 Mean (SD)</th>
<th>Decrease (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I Beads</td>
<td>9.37 (0.101)‡</td>
<td>9.31 (0.12)†</td>
<td>0.8</td>
</tr>
<tr>
<td>II Meshwork</td>
<td>6.36 (0.097)‡</td>
<td>6.00 (0.18)‡</td>
<td>5.66</td>
</tr>
<tr>
<td>III Sr link</td>
<td>9.03 (0.091)‡</td>
<td>8.95 (0.10)†</td>
<td>0.88</td>
</tr>
<tr>
<td>IV Alloy primer</td>
<td>6.13 (0.071)‡</td>
<td>6.01 (0.16)‡</td>
<td>1.95</td>
</tr>
<tr>
<td>V Beads+Sr link</td>
<td>12.63 (0.088)‡</td>
<td>10.90 (0.43)‡</td>
<td>13.69</td>
</tr>
<tr>
<td>VI Beads+alloy primer</td>
<td>6.36 (0.110)‡</td>
<td>5.61 (0.43)</td>
<td>11.79</td>
</tr>
<tr>
<td>VII Meshwork+Sr link</td>
<td>13.15 (0.107)‡</td>
<td>11.06 (0.48)‡</td>
<td>15.96</td>
</tr>
<tr>
<td>VIII Meshwork+alloy primer</td>
<td>7.93 (0.090)‡</td>
<td>6.09 (0.11)‡</td>
<td>23.07</td>
</tr>
</tbody>
</table>

**TC 0 – THERMOCYCLE 0, TC 500 – THERMOCYCLE 500**

The Duncan grouping is represented as alphabets in superscript. Different alphabets denote that values are significant at 5% level.

**TABLE 2** The Two-way ANOVA results eight groups.

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retention methods</td>
<td>531.836</td>
<td>7</td>
<td>75.977</td>
<td>1588.983</td>
<td>0.000</td>
</tr>
<tr>
<td>Thermal cycle</td>
<td>18.780</td>
<td>1</td>
<td>18.780</td>
<td>392.761</td>
<td>0.000</td>
</tr>
<tr>
<td>Retention groups x Thermalcycle</td>
<td>15.948</td>
<td>7</td>
<td>2.278</td>
<td>47.648</td>
<td>0.000</td>
</tr>
<tr>
<td>Residual</td>
<td>3.825</td>
<td>80</td>
<td>0.048</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>570.389</td>
<td>95</td>
<td>6.004</td>
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</tr>
</tbody>
</table>

**TABLE 3** One-way ANOVA of the groups at Thermocycle 0

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Between groups</td>
<td>321.9557</td>
<td>7</td>
<td>45.9937</td>
<td>5086.154</td>
<td>0.0000</td>
</tr>
<tr>
<td>Within groups</td>
<td>0.3617</td>
<td>40</td>
<td>0.0090</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>322.3174</td>
<td>47</td>
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**TABLE 4** One-way ANOVA of the groups at Thermocycle 500

<table>
<thead>
<tr>
<th>Source</th>
<th>Sum of squares</th>
<th>df</th>
<th>Mean square</th>
<th>F</th>
<th>P value</th>
</tr>
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<tbody>
<tr>
<td>Between groups</td>
<td>225.8282</td>
<td>7</td>
<td>32.612</td>
<td>372.590</td>
<td>0.0000</td>
</tr>
<tr>
<td>Within group</td>
<td>3.4634</td>
<td>40</td>
<td>0.0866</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>229.2917</td>
<td>47</td>
<td></td>
<td></td>
<td></td>
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</tbody>
</table>

**TABLE 5 PAIRED’T’TEST**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Mean (SD)</th>
<th>SE of mean</th>
<th>df</th>
<th>t-value</th>
<th>P value</th>
</tr>
</thead>
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<tr>
<td>I</td>
<td>0.0633(0.207)</td>
<td>0.084</td>
<td>5</td>
<td>0.75</td>
<td>0.486</td>
</tr>
<tr>
<td>II</td>
<td>0.3633(0.253)</td>
<td>0.104</td>
<td>5</td>
<td>3.49</td>
<td>0.017</td>
</tr>
<tr>
<td>III</td>
<td>0.800(0.90)</td>
<td>0.037</td>
<td>5</td>
<td>2.17</td>
<td>0.082</td>
</tr>
<tr>
<td>IV</td>
<td>0.1267(0.207)</td>
<td>0.085</td>
<td>5</td>
<td>1.50</td>
<td>0.195</td>
</tr>
<tr>
<td>V</td>
<td>1.7367(0.446)</td>
<td>0.182</td>
<td>5</td>
<td>9.53</td>
<td>0.000</td>
</tr>
<tr>
<td>VI</td>
<td>0.7583(0.482)</td>
<td>0.197</td>
<td>5</td>
<td>3.86</td>
<td>0.012</td>
</tr>
<tr>
<td>VII</td>
<td>2.10(0.503)</td>
<td>0.205</td>
<td>5</td>
<td>10.23</td>
<td>0.000</td>
</tr>
<tr>
<td>VIII</td>
<td>1.8483(0.187)</td>
<td>0.076</td>
<td>5</td>
<td>24.25</td>
<td>0.000</td>
</tr>
</tbody>
</table>

* - SIGNIFICANT DIFFERENCE
** - HIGHLY SIGNIFICANT
NS - NOT SIGNIFICANT